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Abstract

This experiment was designed to study the effects of intensity of exercise on bone adaptations in ovariectomized and sham operated 12 week old rats. Eighty Sprague Dawley rats were divided by mass into two equal groups (mean = 197 g). One group was ovariectomized (OVX); the other sham (S) operated. Each surgery group was then subdivided by mass into four exercise intensity groups. The exercise intensity groups were created by loading additional mass (percent of animals body mass, 0%, 3%, 6% and 9%) on each animal in combination with treadmill running (10 m/min.; 30 min./day; 4 days/wk.; for 7 weeks). Intensity of exercise and OVX had a significant effect on bone integrity (BI) ($P<.001$), a construct that consisted of bone breaking strength, apparent bone density and % ash wt. OVX seemed to influence BI more than intensity of exercise. Breaking strength was significantly affected by OVX ($P<.001$). Upon further analysis, OVX had the most significant effect on breaking strength and ASH% ($P=.023$; $P=.000$; respectively) whereas intensity of exercise did not have a significant effect on any of the construct variables. When the construct variables were compared between groups, bone breaking strength was significantly greater in the OVX than the S ($P=.024$). There was no effect on bone density between the groups, and ASH% was significantly less in the OVX than the S.

Introduction

The magnitude of the effects of involutional or osteoporotic bone loss in postmenopausal women is devastating. Osteoporosis affects half the female population over age 45. By age 75, nine out of ten women are afflicted. Bone loss compromises the integrity of the skeletal structure leaving it most vulnerable to spontaneous fractures of the vertebrae, wrist and hip. In 1992 the U.S. estimated annual cost of hip fractures was 7.3 billion dollars in direct costs with a total of 12 billion when indirect costs were included (Clark, 1992). The monetary costs are exorbitant, but they pale when compared to the personal cost of this disease.

There is a 12-20% mortality rate among the 250,000 hip fractures annually (Levin, 1991). Of those patients who survive, about 50% will dramatically change their lifestyles as a result of loss of mobility. The ability to live independently often ends, and they become nursing home bound (Levin, 1991). Despair and a loss of the will to live may develop as a result of diminished quality of life. Mortality, morbidity, and monetary loss, coupled with psycho-social issues as a result of osteoporosis, are devastating.

Reaching peak bone mass with as much bone as possible and maintaining that mass can help prevent early onset of osteoporosis. According to Papazian (1994), estrogen in combination with a growth hormone is responsible for bone growth in young women during puberty, and it is irrefutable that estrogen deficiency in women results in bone loss. The need for estrogen to maintain bone mineral density cannot be completely abated by exercise. Substantial research, however, supports exercise as a means of increasing bone integrity. According to Frost (1997) and Skerry, (1997) bone strain magnitude and rate of change in strain magnitude are the two most important exercise factors. Frost (1997) quantifies the strain magnitude necessary to elicit bone modeling (adults 800-1200 microstrains; youth 2000-4000 microstrains). Bone modeling dictates bone strength because it influences the amount of tissue and its architecture. Decreased estrogen levels increase the threshold for bone modeling to occur (Westerlind et al., 1997). The definition of an exercise protocol that best promotes bone modeling so that individuals reach peak bone mass and maintain that mass could help facilitate the reduction of osteoporotic injuries.

Materials and Methods

Eighty female Sprague Dawley rats with a mean mass of 197 grams (approximately 12 weeks of age) were used to

conduct this seven week exercise intervention study. The animals were massed and divided into two groups of equal mean mass. One group was ovariectomised (O) the other sham (S) operated.

After surgery, the animals were given a 10-day recovery period. Thereafter, all animals started treadmill training at a speed of 10 meters per minute for 5 minutes. Appropriate additional mass was added to each animal (extra mass pouch with lead shot) to correspond with their intensity grouping. The four levels of intensity groupings were accomplished by adding 0%, 3%, 6% and 9% of the animals' body mass. The initial training run was 5 minutes. The duration of subsequent exercise bouts was increased by two minutes until the animals could run for 15 minutes. Exercise period length was then increased by 5 minutes after each two bouts until the animals could run for 30 minutes. The animals were weighed once a week, and the incremental mass gain of the animal was reflected by additional mass being placed in the extra mass pouch. This additional mass corresponded to the appropriate exercise intensity percentage. The animals exercised 4 days per week, 2 consecutive days with one or two days between bouts for 7 weeks. Throughout the study the animals were allowed to eat and drink ad libitum.

At the end of the seven week training period, the animals were euthanised and the right femur was resected. Bone density (d=m/v), anthropometric measurements (femur length, epiphyseal plate width, mid-shaft width, beginning body mass, ending body mass, mass gain), breaking strength (810 Material Test System (MTS) actuator descent rate .1 mm per sec) and bone mineral ash (ashing protocol: Association of Official Analytical Chemists) were collected.

The experimental design was a 2X4 factorial with the two groups being ovariectomized (OVX) and sham (S) operated Sprague Dawley female rats. The factor was intensity of training, altered by adding one of four levels of additional mass (0%, 3%, 6% and 9% of animals body mass). The groups are differentiated as OVX0, OVX3, OVX6 or OVX9 and S0, S3, S6 or S9 to indicate intensity levels.

Results

Bone Integrity Construct.--A bone integrity construct (BI) was developed by grouping bone breaking strength, bone density and ASH%. The three variables were massed equally. The BI was analyzed using SPSS Multiple Analysis of Covariance (MANCOVA) with final body mass being the covariate. Individual analyses of variance (ANOVA) were calculated for bone breaking strength, ASH% and bone density with respect to surgery and intensity of exercise. Descriptive statistics and correlations were calculated for the dependent variables.

When BI and intensity of exercise and surgery were compared, Wilks multivariate test yielded a value of .61989 which was significant ($P<.001$). Surgery had more influence on BI than exercise intensity although in combination they significantly affected BI. Further analysis suggested that ovariectomy significantly influenced BI and two of the dependent variables, breaking strength and ASH% ($P = .023$; $P<.001$ respectively). Intensity of exercise had no significant influence on BI or the variables individually.

Individual Bone Variables.--The results of the statistical analyses are shown in Tables 1, 2 and 3. Bone density for S rats when compared by intensity of exercise (0%, 3%, 6%, 9%) was not significantly different. Mean bone density of the S rats exhibited a numerically curvilinear trend to exercise intensity (S0, 1.62g; S3, 1.64g; S6, 1.70g and S9, 1.54 g). Bone density within the OVX group did not differ. When bone density was compared between the S and OVX groups, the OVX bone density was 3.9% less than that of the S animals (1.55g and 1.61g, respectively) but was not statistically different. An ANOVA was computed in order to further clarify the effects of intensity of exercise and surgery on bone density (Table 3). Neither intensity of exercise nor surgery influenced bone density.

In both the S and OVX groups, bone breaking strength did not follow a linear path relative to intensity of exercise. Bone breaking strength in the OVX animals was significantly higher than that of the S animals (157.5 n and 146.3 n, respectively; $P = .024$). The correlations suggest that bone anthropometric measures and animal mass were variables that affected bone breaking strength (Table 1 and 2). The combined effects of intensity of exercise and surgery or intensity alone did not influence bone breaking strength (Table 3).

ASH% was significantly influenced by the main effects of intensity and surgery. When considered individually, surgery produced a significant effect and intensity of exercise had no effect (Table 3). ASH % of the femur was significantly greater in the S than OVX group (.6918 g and .6722 g, respectively; $P\leq.001$). The .0196 g difference in Ash represents a 2.9% greater bone mineral content in the S group when compared to the OVX group.

Bone Anthropometric Measurements.--Femoral length, mass and volume further define bone characteristics with respect to exercise intensity and ovariectomy. Femoral length was significantly greater in the OVX group than the S group, a difference of 2.89% (36.66 mm vs. 35.63 mm; $P \leq.001$). Femoral length had a significant positive correlation to bone breaking strength in both the OVX and S groups (Tables 1 and 2). Mass had a greater relationship to femoral length for the S group than the OVX group (Table 1 and 2). ASH% also had a significant positive correlation to femoral length for the S group but not the OVX group.

Table 1. Correlation matrix for breaking strength, body mass plus mass, femur volume, femur mass, initial mass, femur length, ash%, final mass and mass gain in S rats

	Breaking Strength (n/m^2)	Body mass plus mass (g)	Femur volume (ml)	Femur mass (g)	Initial mass (9g)	femur length (mm)	Ash % (g)	Final mass (g)	Mass gain (g)
Breaking strength	1.00	.62 P=.000	.29 P=.123	.81 P=.000	.54 P=.003	.58 P=.001	.38 P=.048	.70 P=.000	.60 P=.001
Body mass plus mass	.62 P=.000	1.00	.48 P=.007	.56 P=.001	.60 P=.000	.61 P=.000	.46 P=.015	.91 P=.000	.82 P=.000
Femur volume	.29 P=. 123	.48 P=.007	1.00	.51 P=.004	.28 P=.135	.12 P=.531	.33 P=.091	.40 P=.028	.35 P=.058
Femur mass	81 P=.000	.56 P=.00 I	.51 P=.004	1.00	.47 P=.009	.60 P=.001	.41 P=.029	.55 P=.003	.39 P=.033
Initial mass	.54 P=.003	.60 P=.000	.28 P=.135	.47 P=.009	1.00	.59 P=.001	.24 P=.217	.69 P=.000	.26 P=.167
Femur length	.58 P=.001	.61 P=.000	.12 P=.531	.60 P=.001	.59 P=.001	1.00	.41 P=.033	.60 P=.001	.42 P=.024
Ash %	.38 P=.048	.46 P=.015	.33 P=.091	.42 P=.029	.24 P=.217	.41 P=.033	1.00	.46 P=.014	.47 P=.013
Final mass	.70 P=.000	.91 P=.000	.40 P=.028	.53 P=.003	.69 P=.000	.60 P=.001	.46 P=.014	1.00	.88 P=.000
Mass gain	.60 P=.001	.82 P=.000	.35 P=.058	.39 P=.033	.26 P=167	.42 P=.024	.47 P=.013	.88 P=.000	1.00

Table 2. Correlation matrix for bone density, breaking strength, femur volume, femur mass, femur length and initial mass in OVX rats.

	Bone Density (wt/vol)	Bone Strength (n/m^2)	Femur Volume (ml)	Femur Mass (g)	Femur Length	Initial Mass (ml) (g)
Bone Density	1.00	.04 P=.820	-.77 P=.000	.06 P=.748	.29 P=.134	-.11 P=.575
Bone Strength	.04 P=.820	1.00	.43 P=.018	.78 P=.000	.48 P=.010	.53 P=.003
Femur Volume	-.77 P=.000	.43 P=.018	1.00	.58 P=.001	.11 P=.580	.39 P=.033
Femur Mass	.06 P=.748	.78 P=.000	.58 P=.001	1.00	.67 P=.000	.49 P=.006
Femur Length	.29 P=.134	.48 P=.010	.11 P=.580	.67 P=.000	1.00	.11 P=.597
Initial Mass	-.13 P=.575	.53 P=.003	.39 P=.033	.49 P=.006	.11 P=.597	1.00

Journal of the Arkansas Academy of Science, Volume 53 [1999], Art. 18

Table 3. The effects of exercise intensity and ovariectomy on bone density, ash %, and breaking strength

	Bone Density (wt./vol.)	Ash% (g)	Breaking Strength (n/m^2)
Main Effect	F = .97 P = .431	F = 11.81 P = .000	F = 1.30 P = .284
Intensity of Exercise	F = .25 P = .856	F = 2.48 P = .072	F = .13 P = .943
Surgery	F = 3.03 P = .088	F = 37.69 P =. 000	F = 4.89 P = .032
Interactions Intensity Surgery	F = .61 P = .613	F = 1.96 P = .131	F = .25 P = .864

F = ratio of the mean square regression to the mean square residual

There was not a significant difference between OVX and S group femoral mass (.9519 g and .9224 g, respectively). As with femur length, body mass variables seemed to relate more to femur mass within the S group than the OVX group. In both S and OVX groups, bone breaking strength was significantly related to femur mass.

Femur volume was significantly higher within the OVX compared to the S group (.6210 ml, and .5773 ml, respectively; P =.014). Intensity of exercise did not significantly influence femoral volume in the S or OVX groups. Within the OVX group, bone breaking strength had a significant positive relationship to femoral volume, which was not the case within the S group (Tables 1 and 2).

Body Mass Measurements.--Body mass appeared to influence bone variables within the S group to a greater extent than the OVX group (Tables 1 and 2). The initial mass of the OVX group was significantly greater than the S group (242.6 g and 233.2 g OVX and S respectively; P = .001). Mass gain between the two groups was significantly different. The OVX group gained an average of 114.63 g compared to the S group that gained only 62.20 g (P< .001).

Discussion and Conclusions

The evaluation of the effects of exercise intensity on bone adaptation is necessary for the development of an exercise protocol that promotes bone growth and lifelong maintenance. The discussion of data centers upon BI, and the individual variables that comprise this construct: bone breaking strength, bone density and bone ash. These variables indirectly reflect bone adaptation.

The reduction of bone mass to the point that minimal stress can produce a fracture (fracture threshold) defines the development of osteoporosis (Aloia, 1989). Bone mass includes both organic (collagen matrix, water, cells, etc.) and inorganic (mineral) components. A major component of bone mass that influences fracture threshold is mineral content and the quality of mineralization. Bone mass and mineralization, however, are not the only factors that influences bone fracture threshold.

Bone architecture plays a significant role in bone integrity. Bone size, shape, cortical thickness as well as distribution of cortical and trabecular bone in the cross-section influence bone strength (Frost, 1997). The loss of cross-bracing trabeculae or the thinning of intact trabeculae compromise the integrity of bone. Mechanical stress and ovarian hormones affect both bone mass and architecture. There are other variables that influence bone integrity, but the scope of this research was limited to the effects of varied mechanical stress (exercise intensity) and ovarian hormone deficiency (ovariectomy) on bone adaptations.

Bone Breaking Strength.--In this study femoral bone breaking strength was significantly higher within the OVX group than the S group. Pohlman et al. (1986) found tensile strength of femoral shaft to be higher within ovariectomized exercising or sedentary groups than their non-ovariectomized counter parts. In contrast, Peng et al. (1994) found that ovariectomized animals experienced a decrease in maximal failure load for the femoral neck. Breaking strength measures within this study were taken as compressive force on the medial shaft of the femur (cortical bone), and Peng et al. (1994) measured breaking strength of the femoral neck (trabecular bone). An explanation for the difference in findings between Peng et al., Pohlman et al., and this study may be due to bone type differences. Trabecular bone constitutes a major portion of the femoral neck while cortical bone is the primary component of the femoral shaft. The mechanical properties of the different bone types impart significant breaking strength differences.

Trabecular and cortical bone respond differently to estrogen deficiency. Most studies indicate that trabecular bone decreases in mineral and matrix and mechanical integrity more rapidly than cortical bone (Iwamoto et al., 1998; Tamaki et al., 1998; Westerlind et al., Gilsanz et al., 1995; Hodgskinson and Currey, 1993; Riggs et al., 1986). More specifically the absence of ovarian hormones can cause a decrease in crossbracing trabeculae, which subsequently will result in decreased breaking strength (Aloia, 1989). The effect of estrogen deficiency on cortical bone is much less severe. Extensive metabolic changes in cortical bone which decrease breaking strength are observed only after long term deficiency, 18 to 26 weeks (Yamazaki and Yamaguchi, 1989; Jee et al., 1991); however, changes occur in trabecular bone between 3 and 4 weeks after ovariectomy (Yamazaki and Yamaguchi, 1989; Jee et al., 1991).

The age of a rat at the time of ovariectomy influences body size and other anthropometric characteristics. Femur length and volume are significantly increased in rats that are ovariectomized at 4 and 10 weeks of age compared to rats ovariectomized at 52 weeks of age (Yamazaki and Yamaguchi, 1989). The rats within this study were ovariectomized at 13 weeks. Similar to findings by Yamazaki (1989) the ovariectomized rats in this study were significantly larger than the sham rats not only in mass but femoral length and volume. This could elucidate the significantly higher femoral breaking strength in the OVX rats when compared to the Sham rats. The continued physiologic bone growth of the younger rats in the absence of ovarian hormones seems to be the stimulus for enhanced femoral growth (Yamazaki and Yamaguchi, 1989; Turner et al., 1987; Jee et al., 1991).

Bone Density.--When bone density was compared between OVX and S groups, the OVX group was numerically less but there was no significant difference. This was contradictory to findings by Iwamoto et al. (1998), Tamaki et al. (1998) and Westerlind et al. (1997). The previously mentioned studies all found significant reduction in trabecular bone area specifically within certain regions of long bone. They found reduction of bone area in both the proximal and distal regions with most loss occuring in the central region of the cancellous metaphysis. The difference found in BMD in this study could possibly be explained by differences in BMD measurements (calculated vs histomorphometric)

Although exercise intensity had no significant effect on BMD within this study, numerical differences were observed. Because the degree to which an increase/decrease in BMD affects structural integrity is not known, a statistically non-significant increase may very well have physiological pertinence. For this reason, the numerical trends will be discussed. Mean bone density within the S groups increased from S0 through S6 with a decrease from S6 to S9. Exercise has been demonstrated by some researchers to augment bone mass (Iwamoto et al., 1998; Tamaki et al., 1998; Westerlind et al., 1997; Dalsky et al. 1988; Grove and Londeree, 1992; Ayalon et al., 1987), while other researchers have observed loss of bone as one of the consequences of over-training (Iwamoto et al., 1998; Drinkwater et al., 1984; Michel et al., 1989). Iwamoto et al. (1998) found that duration of a moderate intensity exercise had a significant effect on BMD. Iwamoto, compared 30 minutes of treadmill running to 60 minutes of running (same intensity 16 m/min) in OVX rats. He found a significant increase in bone area in both trabecular and cortical bone within the exercising 30 minute group but not in the exercising 60 minute group. Such findings substantiate an overtraining effect and indicate that certain ranges of intensity and duration create an osteogenic effect.

The OVX group did not respond to intensity of exercise in the same manner as the Sham group. Between OVX0 and OVX3 there was a decrease in bone density, but an increase occurred from OVX3 through OVX9. The increase in bone density between the OVX3 and OVX9 groups was significant. The animals comprising the OVX3 group were lighter than the animals in the OVX0 group. The OVX0 animals had a larger bone density than OVX3 animals. This may be due to body mass and its direct effect on the level of mechanical strain when the heavier animals exercised. Westerlind et al. (1997) postulated that estrogen deficiency would increase the threshold at which bone cells would respond to mechanical strain. If that hypothesis is correct then bone cells subjected to the lowest mechanical strain are at the greatest risk of being resorbed. Low strain level could explain the differences found between OVX0 and OVX3. The numerically linear increase in bone density observed from 3% to 9% intensity may suggest that without the benefit of estrogen, the greater mechanical strain placed on the bone did promote bone modeling. Bone density did not correlate to breaking strength for either group. Because in this study bone density is a calculated value, it does not reveal the way in which mineral/matrix is specifically distributed throughout the bone. More sensitive measures which depict bone architecture (histological determination of trabecular bone volume and trabeculae arrangement) might have revealed differences due to exercise intensity. Some researchers have reported differences in trabecular bone morphology as a result of OVX in as little as 3 to 4 weeks (Yamazaki and Yamaguchi, 1989), while others have observed differences as a result of exercise at six weeks (Peng et al., 1994).

Percent Ash.--Ovariectomy had a significant effect on ASH% ($P<.001$) with the OVX group having significantly less bone ash than the S group. This is in accordance with findings by Yamazaki and Yamaguchi (1989), who observed that bone ash in 10 week old rats was significantly affected by ovariectomy. Intensity of exercise did not significantly

affect ASH% but approached significance in both groups. Within the S group, bone ash was significantly correlated to breaking strength, a relationship not observed in the OVX group. Within the OVX group, initial body mass was the only variable that had a relationship to bone ash. Other variables that related to ASH% within the S group were femur mass and length. A deficiency of ovarian hormone apparently results in complex alterations of bone metabolism. This appears to be substantiated by the relationship of such variables as breaking strength, bone ash, femur mass and length observed in S but not OVX rats.

Final body mass plus the final added mass is a variable that reflects intensity of exercise. Within the S group, there was a significant positive correlation between body mass plus mass added and ASH% (P=.015). Because ASH% significantly correlated with breaking strength within the S group, intensity of exercise apparently made a positive contribution to bone adaptation within this group.

Bone integrity was significantly affected by both ovariectomy and exercise intensity, with ovariectomy having the most profound influence. Because of the confounding influence of ovariectomy on bone architecture, it was not possible to determine whether exercise intensity caused any of the differences (numerical or statistical) observed. Further, the construct of BI was also less informative because of this interaction. Therefore, future investigations should focus on minimizing the effects of growth and ovariectomy on data, while pursuing the level of intensity at which bone integrity is optimized.

Although there was no significant effect of intensity of exercise on bone breaking strength, bone density or ASH%, data observed within this study suggest adaptations associated with exercise intensity. For example, ASH% approached significance (P =.072) with respect to exercise intensity in both the S and OVX groups. While bone density in the S group followed a curvilinear path. At the highest level of intensity, decreases in both ASH% and bone density were noted. These findings may indicate a training effect with the drop corresponding to a detrimental over-training effect as indicated previously by others (Iwamoto et al., 1998; Frost, 1997; Michel et al., 1989; Drinkwater et al., 1984).

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